Gene Therapy Targeting Monocyte Chemoattractant Protein-1 for Vascular Disease

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Monocyte chemoattractant protein-1 (MCP-1) has been shown to play an essential role in the pathogenesis of arteriosclerosis and other vascular diseases, such as restenosis after arterial injury, by recruiting monocytes into the arterial wall. We devised a new strategy for anti-MCP-1 gene therapy against arteriosclerosis by transfecting an amino-terminal deletion mutant (7ND), which lacks the amino-terminal amino acids 2 to 8 of the human MCP-1 gene, into a remote organ (skeletal muscles). Intramuscular transduction with the mutant MCP-1 gene suppressed inflammatory and proliferative changes and arteriosclerosis formation induced by the chronic inhibition of nitric oxide synthesis in rats. 7ND gene transfection also inhibited the initiation, progression, and destabilization of arteriosclerosis in Apolipoprotein E-knockout mice. Moreover, the strategy reduced restenosis after balloon injury in rabbits, rats, and monkeys, or neointimal formation after stent implantation in monkeys. This new strategy may be a useful and feasible gene therapy against arteriosclerosis and restenosis after angioplasty. J Atheroscler Thromb, 2002; 9: 261-265.

Key words: Monocyte chemoattractant protein-1, Arteriosclerosis, Restenosis, Gene therapy

Introduction

Leukocyte infiltration has been shown to be present in atherosclerotic lesions. Recent studies suggest that the inflammatory response plays an important role not only in atherosclerosis but also in other cardiovascular diseases, such as restenosis after balloon angioplasty or stent implantation, arteriopathy after organ transplantsations, and vascular remodeling due to hypertension. Chemokines are proinflammatory cytokines, which regulate leukocyte chemoattraction and activation. Migration and infiltration into tissues, and the subsequent activation of leukocytes are regulated by chemokines. In fact, the upregulation of various chemokines in atherosclerotic lesions has been reported (1). About 80% of leukocytes in atherosclerotic lesions are monocytes/macrophages, and 10 to 20% of them are memory T lymphocytes. Monocyte chemoattractant protein-1 (MCP-1) is the most important chemokine which regulates the migration and infiltration of monocytes/macrophages. MCP-1 belongs to CC chemokines, a subfamily of chemokines and is the specific chemotactic factor for monocytes/macrophages, playing an important role in the pathogenesis of chronic inflammation. The effects of MCP-1 are mediated mainly through CC chemokine receptor 2 (CCR2). Recent studies reported that MCP-1 might play an important role in the initiation and pathophysiology of cardiovascular diseases (2,3). These facts suggest that therapeutic strategies targeting MCP-1 may be effective in the treatment of cardiovascular diseases. We have developed a new therapeutic strategy which can inhibit MCP-1 effectively in vivo. In this review, we will describe the role of MCP-1 in cardiovascular diseases and the results of studies evaluating the efficacy of our anti-MCP-1 gene therapy using mutant MCP-1 gene transfection.
The role of MCP-1 in vascular diseases

At present, atherosclerosis is recognized as a chronic inflammatory disease, and the migration of monocytes/macrophages is thought to be an important event which occurs in the early phase of its development. MCP-1 is secreted by activated endothelial cells or smooth muscle cells, and it plays an important role in the migration and infiltration of monocytes/macrophages into the arterial wall (4). Recently, it has been reported that MCP-1 is upregulated in atherosclerotic lesions (5). Boring et al. crossed CCR2-deficient (CCR2-/-) mice with apolipoprotein E-knockout mice which develop severe atherosclerosis, and reported that the selective absence of CCR2 markedly decreases lesion formation in apoE-knockout mice (6). Namiki et al. transfected the cDNA encoding rat MCP-1 into the vessel wall of the rabbit carotid artery using the hemagglutinating virus of Japan (HVJ)-liposome method. They reported that all cholesterol-fed rabbits displayed neointimal formation with the infiltration of RAM-11-positive monocytes, and a part of the lesion also had lipid deposition, although no vascular lesion formation was found in normal chow-fed rabbits (7). These data suggest that MCP-1 plays an important role in the development of atherosclerosis.

Inflammation also contributes to the development of restenosis after angioplasty, and a potent role of MCP-1 in the pathogenesis of restenosis has been suggested. A rapid increase of MCP-1 expression after balloon injury has been shown. Cipollone et al. reported that restenotic patients, compared with nonrestenotic patients, had statistically significant elevated levels of MCP-1 after PTCA and higher MCP-1 throughout the study was correlated with restenosis. Moreover, increased MCP-1 was significantly correlated with increased monocyte activity and the MCP-1 plasma level measured 15 days after PTCA was a statistically significant independent predictor of restenosis (8). Roque et al. tested the effect of CCR2 deficiency in a murine model of femoral arterial injury. They reported a significant reduction in intimal hyperplasia after arterial injury in CCR2-/- mice compared with CCR2+/+ littersmates (9). These data suggest that MCP-1 and its receptor CCR2 may play a pivotal role in the pathogenesis of restenosis after angioplasty (Fig. 1).

Therapeutic strategy targeting MCP-1 using mutant MCP-1 gene transfection

We have recently reported that an N-terminal deletion mutant of human MCP-1 (7ND), which lacks the N-terminal amino acids 2 to 8, acts as a dominant negative inhibitor for MCP-1 and blocks the MCP-1/CCR2 signal pathway in vivo (10). Rollins et al. reported that this mutant MCP-1 binds to the MCP-1 receptor (CCR2) and completely inhibits MCP-1-mediated monocyte chemotaxis in vitro (11) (Fig. 2A). We planned the therapeutic strategy targeting MCP-1 using this mutant MCP-1 gene transfection (Fig. 2B). We transfected the expression plasmid vector encoding 7ND gene into skeletal muscle, and demonstrated that 7ND protein was secreted from the transfected skeletal muscle cells into the circulating blood for at least 2 to 4 weeks, and subsequently blocked monocyte infiltration into the dermis induced by the subcutaneous injection of recombinant MCP-1 (Fig. 2B) (10). On the basis of these results, we investigated the effect of this strategy on several models of vascular disease, and report the data in this review.

Effect of 7ND gene transfection in models of vascular diseases

1. Arteriosclerotic rat as a model of chronic inhibition of nitric oxide synthesis

We have reported that the chronic inhibition of nitric oxide synthesis by the administration of N’-nitro-L-arginine methyl ester (L-NAME) induces early inflammation [monocyte infiltration, monocyte chemoattractant protein-1 (MCP-1) expression, nuclear factor-κB (NF-κB) activation] and late cardiovascular remodeling (medial thickening, perivascular fibrosis, and cardiac fibrosis) in rats (12-14). 7ND gene transfection almost completely suppressed the

![Diagram](image_url)

**Fig. 1.** The role of MCP-1 in the pathogenesis of atherosclerosis and restenosis after balloon injury.
inflammatory and proliferative changes (increase of ED1 positive monocytes/macrophages and PCNA-positive cells) on day 3 of L-NAME administration (Fig. 3A) (10). We also tested the effect of 7ND gene transfection on cardiovascular remodeling (Fig. 3B). This strategy reduced the development of vascular medial thickening, but not perivascular fibrosis (10). Thus, MCP-1 is necessary for the development of medial thickening but not of fibrosis in this model. These data suggest that MCP-1 plays an important role in the pathogenesis of early cardiovascular inflammatory and proliferative changes, and late atherosclerosis formation in this model. On the other hand, 7ND transfection inhibited neither TGF-β expression nor hypertension, thus these factors, other than MCP-1, are suggested to participate in the pathogenesis of fibrosis in this model.

2. Atherosclerotic ApoE-knockout mice model
ApoE-KO mice spontaneously develop hypercholesterolemia and atherosclerotic lesions similar to those found in humans and are widely used to study the pathogenesis of atherosclerosis (15,16). We transfected the 7ND gene to ApoE KO mice at 7 to 8 weeks of age, which have not developed apparent atherosclerotic lesions, and determined the effect of the transfection on atherosclerosis formation after high cholesterol diet administration. Blockade of the MCP-1 pathway inhibited the formation of atherosclerotic lesions but had no effect on serum lipid concentrations (17) (Fig. 4A, 4B, 4C). Furthermore, this strategy increased the lesional extracellular matrix content and accordingly, the plaque stability score (Fig. 4D). These results suggest that MCP-1 is associated with not only atherogenesis but also with vulnerable atheromatous plaque stabilization. We also determined the effect of MCP-1 blockade on the progression of pre-existing atherosclerotic lesions in the aortic root in ApoE KO mice at 20 weeks of age and MCP-1 blockade was able to limit the progression of established lesions. In addition, MCP-1 blockade improved the lesion composition into a more stable phenotype, i.e., containing fewer macrophages and lymphocytes, less lipid, more smooth muscle cells and collagen. This strategy decreased the expression of CD40 and the CD40 ligand in the atherosclerotic plaque and normalized the increased chemokine (RANTES and MCP-1) and cytokine (TNFα, IL-6, IL-1β, and TGFβ-1) gene expression in the abdominal aorta (18).

![Diagram](image_url)

**Fig. 2.** Structure-functional relationships of MCP-1 (A). Therapeutic strategy of mutant MCP-1 (7ND) gene transfection (B). A: 7ND is an N-terminal deletion mutant of human MCP-1, which lacks the N-terminal amino acids 2 to 8, and acts as a dominant negative inhibitor for MCP-1, blocking the MCP-1/CCR2 signal pathway and completely inhibiting MCP-1-mediated monocyte chemotaxis. B: We transfected the expression plasmid vector encoding the 7ND gene into skeletal muscle. 7ND protein is secreted from the transfected skeletal muscle cells into the circulating blood, blocking the MCP-1/CCR2 signal pathway in target organs, and is expected to improve organ dysfunction.

![Diagram](image_url)

**Fig. 3.** Effect of 7ND gene transfection on inflammatory and proliferative changes and cardiovascular remodeling induced by the chronic inhibition of nitric oxide synthesis in rats. A: 7ND transfection inhibited inflammatory changes on day 3 of L-NAME administration. B: 7ND transfection also inhibited medial thickening on day 28 of L-NAME administration.
Fig 4. Inhibition of atherosclerosis formation and lesional macrophage accumulation by 7ND gene transfer.

A: Photomicrographs of aortic atherosclerotic lesions stained with oil red O and MOMA-2 (monoclonal antibody against mouse macrophage/macroplage) from PBS injected and 7ND-transfected ApoE-KO mice. Bars represent 200 μm.

B: Quantitative comparison of atherosclerotic lesion size (oil red O stained area) in PBS injected and 7ND-transfected ApoE-KO mice. *p < 0.05 vs. PBS-injected group. Data are reported as the mean ± SEM, n = 8.

C: Quantitative analysis of the macrophage area in atherosclerotic lesions from PBS-injected and 7ND-transfected ApoE-KO mice. *p < 0.05 vs. PBS-injected group. Data are reported as the mean ± SEM, n = 7 to 8.

D: Plaque stability score [α-SM actin positive area + collagen area]/(macrophage area + oil red O area) of lesions from PBS-injected and 7ND-transfected ApoE-KO mice. *p < 0.05 vs. PBS-injected group. Data are reported as mean ± SEM, n = 7 to 8.

These data suggest that MCP-1 is a central mediator in the progression and destabilization of established atheroma and the inflammatory responses mediated by MCP-1 are important in atherosclerosis and its complications.

3. Restenosis models after balloon injury or stent implantation

We also tested the hypothesis that 7ND gene transfection inhibits vascular restenosis after balloon injury or stent implantation. We determined the effect of 7ND transfection on the development of restenotic changes after balloon injury in the carotid artery in hypercholesterolemic rabbits. We found that, after balloon injury, the MCP-1 mRNA level significantly increased and the appearance of RAM11-positive macrophages became evident on days 3 to 7, and neointimal formation and negative remodeling (smaller lumen size, internal elastic lamina and external elastic lamina) on day 28. Intramuscular transfection of the 7ND gene suppressed monocyte infiltration/activation in the injured arterial wall and thus attenuated the development of neointimal hyperplasia as well as negative remodeling (19).

We also investigated whether this anti-MCP-1 strategy might be effective in reducing neointimal hyperplasia after carotid artery balloon injury in other animals including primates.

We observed increases in the gene and protein expression of MCP-1, infiltration of monocytes into the intima, and the appearance of proliferating cells in the early stages (days 3 to 7) of balloon injury in rats. Neointimal hyperplasia was evident in the later stage (day 28). Transfection of the mutant MCP-1 gene suppressed such early inflammatory and proliferative changes and inhibited neointimal hyperplasia by 60%. Furthermore, this strategy reduced neointimal hyperplasia after balloon injury by 70% and intimal thickening after stent implantation in monkeys.

These data suggest that MCP-1-mediated monocyte infiltration is essential in the development of neointimal hyperplasia after balloon injury and stent implantation in rats and monkeys. This strategy may be a useful form of gene therapy against human restenosis after percutaneous coronary intervention.

Conclusion

MCP-1 plays a pivotal role in chronic inflammation in cardiovascular disease, such as atherosclerosis and restenosis after vascular injury, by regulating monocyte/macrophage migration and activation. The new therapeutic strategy, described in this review, may be a useful and feasible gene therapy against atherosclerosis or restenosis after coronary intervention in humans. Recently, we demonstrated that no significant side effects associated with 7ND transfection were found. We are planning to apply this strategy to clinical restenosis after percutaneous coronary intervention, and this application is under deliberation in the Ministry of Health, Labor and Welfare of the Japanese government.

References


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